



(11) Publication number: **0 309 464 B1**

(12) **EUROPEAN PATENT SPECIFICATION**

(45) Date of publication of patent specification: **09.12.92** (51) Int. Cl.<sup>5</sup>: **A61K 9/50, A61K 9/12**

(21) Application number: **87903720.8**

(22) Date of filing: **05.06.87**

(86) International application number:  
**PCT/GB87/00391**

(87) International publication number:  
**WO 87/07502 (17.12.87 87/28)**

The file contains technical information submitted  
after the application was filed and not included in  
this specification

(54) **MEMBRANE LIPID COMPOSITION AND METHOD FOR ITS PREPARATION.**

(30) Priority: **06.06.86 GB 8613811**

(43) Date of publication of application:  
**05.04.89 Bulletin 89/14**

(45) Publication of the grant of the patent:  
**09.12.92 Bulletin 92/50**

(84) Designated Contracting States:  
**AT BE CH DE FR GB IT LI LU NL SE**

(56) References cited:  
**EP-A- 0 087 993**  
**EP-A- 0 229 561**  
**US-A- 3 594 476**

(73) Proprietor: **PHARES PHARMACEUTICAL RE-  
SEARCH N.V.**

**John B. Gorsiraweg no 6 P.O. Box 889**  
**Curacao Netherlands Antilles(AN)**

(72) Inventor: **LEIGH, Steven**  
**6th Floor 63 Lincoln's Inn Fields P.O. Box 22**  
**London WC2A 3JU(GB)**

(74) Representative: **Cole, Paul Gilbert et al**  
**BERESFORD & Co 2-5 Warwick Court High**  
**Holborn**  
**London WC1R 5DJ(GB)**

**EP 0 309 464 B1**

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid (Art. 99(1) European patent convention).

## Description

This invention relates primarily to a composition based on a membrane lipid and at least one biologically active compound in the form of a micronised powder and to a method of making a progenator  
5 of liposomes or proliposome in the form of an aerosol or discrete particles.

Membrane lipids are lipids which form bilayers with water; they are chiefly phospholipids such as lecithin and related materials such as glycolipids. Phospholipid vesicles are also known as liposomes. In addition to naturally occurring membrane lipids, synthetic bilayer-forming materials may also form similar  
10 vesicles known as niosomes. According to the general nomenclature, all types of lipid bilayers surrounding an aqueous space are generally known as liposomes which term shall include niosomes. The promise held out by liposomes as a means of delivering and targeting drugs has, not surprisingly, prompted intensive research into this subject.

Our European Patent Application No. 85301602 (Publication No EP-A-0158441) describes pro-liposome compositions that spontaneously form vesicles or liposomes in the presence of excess water and that may  
15 be presented inter-alia in sprayable form. Thus there are provided aerosol compositions comprising in a volatile liquid propellant:-

- (a) at least one membrane lipid;
  - (b) at least one water-miscible liquid which is a solvent for the lipid; and
  - (c) up to 40%, by weight on the combined weights of a), b) and c), of water;
- 20 the proportion by weight of a) to b) being from 40:1 to 1:20.

This invention is based on the realisation that sprayable compositions or compositions in the form of discrete particles containing a bilayer lipid and a biologically active compound in the form of a micronised powder combine high initial entrapment of the active compound in the lipid with sustained release at the site  
25 of application. The invention provides compositions, methods and a use as set out in the claims.

### AEROSOL COMPOSITIONS

It has been found that pro-liposomes may be made in the form of aerosols of at least one membrane lipid and at least one biologically active compound, the aerosol being free from other solvents. Further  
30 advantages of this type of composition include freedom from unnecessary solvents or water, more control over particle size and improved stability.

A preferred method of forming the pro-liposome aerosols is by spraying under pressure a single composition which comprises at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is  
35 present as a micronised powder dispersed in the propellant and/or the lipid, the composition being free from other solvent for the drug.

The invention further provides a sprayable pro-liposome composition comprising at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present as a micronised powder dispersed in the  
40 propellant and/or the lipid, the composition being free from other solvent for the lipid.

It is well known that biologically active compounds can be incorporated into liposomes partly to protect and partly to enhance their activity as mentioned in our European Patent Specification No. A-0158441. One of the features of this invention is the relative ease with which high levels of drug or other biologically active compound can be associated with liposomes.

It has been demonstrated by in-vitro tests that pro-liposome compositions according to the present invention not only enable high initial entrapment of drug, but surprisingly, also provide for sustained release of the drug at the site of application. It has been found quite unexpectedly that compositions according to the present invention are superior to previously known liposome and pro-liposome aerosol compositions in prolonging the action of drugs at the site of action. Accordingly the compositions of (or made according to)  
50 the invention are particularly suitable for bronchial, pulmonary, and nasal inhalation treatments. They are also suitable for oropharyngeal and topical applications where prolonged effects are desirable to minimise frequency of dosage. Combinations of lipid and drug incorporated into the composition will preferably have the proper electrical charges. Most naturally occurring phospholipids are negatively charged. Some, especially those that are electrically neutral, may require addition of minor amounts of compatible organic materials that can confer the required charge on them. A negative charge can be imparted to the lipid  
55 material by adding, for example, phosphatidic acid or dicetyl phosphate, and a positive charge can be imparted by adding an amine such as stearylamine.

The proportion of lipid in the composition is desirably as small as possible (in order to minimise the risk

of clogging the orifices of the spray canister) but concomitant with obtaining the optimum level of drug association. The weight ratio of drug to lipid will depend on the activity of the drug and may typically be from 1:0.5 to 1:25, more usually 1:1 to 1:24 and preferably from 1:6 to 1:12. For highly potent drugs, the appropriate drug/lipid ratio may be above 1:25.

5 The volatile liquid propellant used in the sprayable compositions of the invention may be butane or dimethylether, in circumstances where its use is permitted, but is preferably a perhalocarbon, particularly Arcton 11 (CClF<sub>3</sub>), Arcton 12 (CCl<sub>2</sub>F<sub>2</sub>) or Arcton 114 (C<sub>2</sub>Cl<sub>2</sub>F<sub>4</sub>). The propellant generally constitutes from 75% to 99%, usually from 87% to 97% by weight of the overall composition.

Suitable membrane lipids are phospholipids, for example, phosphatidyl choline, such as soy lecithin and  
 10 egg yolk lecithin and synthetic lecithins, e.g. dipalmitoyl phosphatidyl choline. Other materials such as glycolipids may be used. Other bilayer-forming materials that can be used include long-chain dialkyl dimethyl ammonium compounds, for example di-stearyl dimethyl ammonium compounds such as di-stearyl dimethyl ammonium chloride, di-tallow dimethyl ammonium compounds such as di-tallow dimethyl ammonium chloride and mono- and dialkyl polyoxyethylene derivatives, although possible irritancy may limit their  
 15 use to non-inhalation applications. In this text, the generic term lipid is used to describe all such bilayer-forming compounds.

Most biologically active compounds can be incorporated into the sprayable composition. In most instances the biologically active compounds should be insoluble or only sparingly soluble either in the lipid or in the propellant. Accordingly they are incorporated into the composition in the form of micronised  
 20 powders of mean particle size in the range of from 0.5 micron to 8 microns, preferably from 1 micron to 5 microns.

On being sprayed, e.g. from an aerosol container, the propellant preferentially volatilises, leaving an aerosol of the remaining components as a pro-liposome composition in the form of droplets of a size determined by the spray nozzle and internal pressure and preferably below 8 microns. On contacting water,  
 25 these droplets form a dispersion of liposomes which constitute very effective drug carriers.

#### POWDER COMPOSITIONS

A second method of making the pro-liposome aerosol is from micronised solid particles whose major  
 30 component is a physiologically acceptable solid carrier and whose minor components are the biologically active compound in particulate dispersion in the lipid. Glucose or lactose are suitable solid carriers and being water-soluble, they dissolve on contact with a moist surface such as a biological membrane. However, hydrophilic materials such as polysaccharides (dextrans and starches), polymers (polyethylene), proteins (albumen, casein) and soluble collagen may be suitable for non-inhalation applications as long as they are  
 35 free-flowing micronised powders, and compatible with the lipid.

The weight ratio of membrane lipid to carrier is suitably from 1:1 to 1:25, preferably from 1:5 to 1:20. The ratio of drug to lipid is as before. The powder aerosol may be formed by vibrating or propelling the particles from a capsule (as in a spinhaler type device) into a moving airstream.

It has been found that high levels of entrapment and improved in-vitro release profiles are obtained  
 40 when the biologically active compound is in micronised dispersion in the lipid. Dispersion/suspension of the drug is assisted if the lipid is first dissolved in a volatile solvent which is not a solvent for the drug. Perhalocarbons which are conventionally used as propellants, particularly Arcton 11, are suitable because they are inert and are usually poor solvents for most biologically active compounds. However, depending on the properties of the powder and the drug, other lipid solvents eg. chloroform or dimethyl ether may be  
 45 used. The solid carrier must not be soluble in the solvent. Alcohol or methanol would be less suitable if the drug is substantially soluble.

The solvent is evaporated off completely either before or after addition of the solid carrier. The resulting powder composition comprises a uniform mixture of solid particles whose major component is a physiologically acceptable solid carrier and whose minor components are the biologically active compound in  
 50 particulate dispersion in the lipid.

In contrast, the powder compositions described by Payne et al (Journal of Pharmaceutical Sciences, Vol.75, No.4, April 1986) require the drug to be in solution in the lipid. A 2:1-methanol/chloroform solution of the drug is loaded onto the powder under carefully controlled conditions. It is reasonable to assume that the solid carrier would be evenly loaded with a coating of lipid and drug in solution. They confined their studies  
 55 to coarse powders in the range 75 to 600 microns and concluded that their pro-liposome system was ideally suited for so-called lipophilic actives.

We have found that this method gives rise to compositions which may have high initial association (60% - 70%). However, our in-vitro studies have indicated that they do not retain the biologically active

compound, whose release profile is quite different, and characterised by uncontrolled release of the active compound into the aqueous phase.

The invention will be illustrated by the following examples. Any registered trade marks used in the specification are acknowledged as such.

5

#### EXAMPLE I

##### Sprayable compositions and entrapment of Fenoterol therein

10 The following sprayable compositions were made up using soya bean lecithin (SBL), a 23:54:23 parts by weight mixture of Arcton 114, Arcton 12 and Arcton 11 as propellant and micronised fenoterol as its free base or hydrobromide as biologically active compound.

The lipid was initially dissolved in a small quantity of Arcton 11. Micronised fenoterol was dispersed in this solution. The rest of the propellant mixture was added to the suspension. The composition was filled  
15 into 20 ml aerosol containers fitted with metered heads delivering a nominal 65  $\mu$ l.

The samples were used to form pre-liposome aerosols that were allowed to form liposomes by contact with excess water. The % of fenoterol associated with each liposome dispersion was evaluated by the procedure described below.

The aerosol pro-liposome compositions of samples 1 to 5 were sprayed from canisters which contained  
20 no dip tube into a phosphate buffer solution (pH7). One gram of propellant/drug/lipid pro-liposome was sprayed into 50 ml of buffer contained in a 250 ml flask adapted to receive the inverted aerosol container above. Liposome suspensions were formed spontaneously as the aerosol mist encountered the buffer solution. The flask was gently swirled to contact the suspended aerosol with the buffer. This process was repeated until the aerosol canister had been drained of one gram of its contents. Fenoterol was assayed as  
25 follows. The fenoterol/liposome suspension was centrifuged at 180,000 g for 90 minutes. The supernatant was assayed for free drug by measuring absorbance at 276 nm. The liposomal pellet containing trapped fenoterol was resuspended in 1% nitric acid to release bound drug and assayed as before. The percentage of drug associated with the liposomes was calculated accordingly.

The entrapment results for each of the five samples are indicated in Table I below.

30

TABLE I

Sample No	Propellant Wt(g)	Drug Wt(g)	SBL Wt(Mg)	% SBL Associated Initial Entrapment	
				HBr	Base
1	9	50	50	15	15
2	9	50	150	42	35
3	9	50	300	60	45
4	9	50	600	75	57
5	9	50	1200	85	72

#### 45 EXAMPLE II

##### Investigation of the effects of lipid-drug ratio on sustained release properties in-vitro

Factors relevant to the sustained release of fenoterol in-vitro were investigated as follows. Fixed weights  
50 of aerosol were sprayed into a large conical flask containing 50 ml of phosphate buffer. The flask was vented at frequent intervals to prevent the build up pressure from vapourised propellant. The final concentration of fenoterol hydrobromide in the test suspension was normally about 0.5 mg/ml but measurements can be made using drug concentrations as low as 0.05 mg/ml. The test suspension was incubated at 37°C. Samples were removed at intervals and centrifuged for 30 min at 175,000xg to pellet the  
55 lipid fraction. The concentration of fenoterol in the supernatant fraction was then measured by HPLC. The percentage of drug retained in the lipid fraction was calculated by comparison with the total fenoterol content of a similar non-centrifuged sample in which the lipid had been solvated in excess methanol. This data was then used to plot delayed release profiles of the different samples.

The effects of altering the ratio of standard lipid I (95% pure soya bean lecithin) to fenoterol hydrobromide on the release profiles of our samples are illustrated in Figure 1 in which percentage retention of fenoterol is plotted as a function of time in hours for so-called Type I aerosols prepared from standard lipid I with lipid:drug ratios of 12:1 (circles), 6: 1 (squares) and 3:1 (triangles). The descending form of the curves denotes an initial high association between the fenoterol and the lipid phase, a subsequent slow release of part of the fenoterol into the aqueous medium and an eventual steady state determined by the partition coefficient between aqueous and lipid phases. The main effect of increased lipid content, apart from changing the final equilibrium position, was to alter the proportion of drug released within the first 30 min after spraying. Unfortunately, the time involved in sample preparation and centrifugation precluded an analysis of the release characteristics over this period. However, the results suggested that there was an initial very rapid release from solvated droplets followed by a slower release over a period about three hours before equilibrium was reached. This was more clearly demonstrated in the three semi-logarithmic plots of percentage retention against time for the different samples presented in Figure 2.

### EXAMPLE III

#### Effect of Lipid Modifiers

The inclusion of materials that might be expected to affect the lipid phase was investigated in-vitro. The effects of including cholesterol (33 mol %) and the saturated triglyceride glycerol tripalmitate (33 wt. %) in 12:1 lipid/drug formulations was investigated by determining percentage retention of fenoterol as a function of time for Type I aerosols of 12:1 drug/lipid ratio made from standard lipid, mixtures of standard lipid with cholesterol 33 mol %, standard lipid and glycerol tripalmitate 33 wt % and standard lipid and SPAN 40 (10 wt %). The effects of adding the surfactant SPAN 40 (10 wt. %) which might be expected to hasten hydration are shown for comparison. In practice, all the modifiers appeared to bring about increased rates of drug release in-vitro. The precise reasons for this are not clear. The most probable explanation, however, is that their different solubilities in the aerosol propellant resulted in the precipitation of the lipid as a phase-separated rather than a homogeneous mixture and that this leads to changes in the structural characteristics in the lipid droplets. However, in certain situations the use of standard modifiers need not be excluded.

### EXAMPLE IV

#### Use of Different Lipids

The effects on sustained release of fenoterol of using two grades of soya bean lecithin (standard lipid I and II); purified egg yolk lecithin (EYL), purified hydrogenated lecithin, EYL + phosphatidyl choline, and EYL + phosphatidyl serine were investigated using the techniques outlined above. Fenoterol was released from such preparations extremely quickly. This extreme leakiness could be associated with the fact that the hydrogenated lipids tend to be in the gel rather than the liquid crystalline state at 37°C. However, addition of cholesterol, which tends to disrupt gel-phase lipid, led to little or no-improvement in the sustained release capacities of the preparations suggesting that other factors may be influencing their properties.

A particularly striking feature of the measurements performed using hydrogenated lipids is the extremely low interaction between the lipid and the drug. Earlier experiments have suggested that the higher the purity of the lipid, the less strongly bound the drug. In order to test whether the lack of binding of the hydrogenated lipids was associated with their hydrogenation or their high purity, we performed a series of experiments using highly purified lecithin (Lipid Products, Grade 1) standard lipid I (95% pure soy-bean lecithin) and another grade of partially purified lecithin (Lipid II). It was clear from measurements obtained that the Lipid I and Lipid II preparations performed better in terms of delayed release than the highly purified lipid. This result probably reflected the presence in the samples of species providing extra drug binding sites. The most likely candidates for such a species were the acidic lipids phosphatidylethanolamine and phosphatidylserine. We therefore prepared mixtures of purified lecithin together with 25 mol% of these lipids. Delayed release profiles of these samples showed that inclusion of the acidic lipids led, in both cases, to an increase in drug binding resulting in higher final equilibrium levels but the effects were more marked for phosphatidylserine than for phosphatidylethanolamine. The presence of these lipids, however, did not seem to delay the initial fast release characteristics of the purified lipid. This result was in agreement with studies using negative amphipaths such as dicetyl phosphate. The inclusion of such materials appeared to increase the partition of fenoterol into the lipid phase giving rise to high initial association but also tended to increase leakage rates.

EXAMPLE VStructure of the Lipid Droplets Delivered by the Aerosols

5 The compositions readily formed liposomes when sprayed onto an aqueous surface. Examination by freeze-fracture electron microscopy revealed a mixture of partially hydrated lipid aggregates and liposomes. The lipid aggregates appeared to be composed of lipid in the process of hydration and were likely to reorganise into liposomes on standing. Figure 3 which is on a scale of 1 cm equals 1.1  $\mu\text{M}$  shows a typical field of liposomal structures formed on spraying a Type I aerosol containing fenoterol into water. The field  
 10 contained liposomes (L) and partially hydrated liquid aggregates (PHA). The EMs shown in Figure 3 clearly showed liposomes in the process of budding-off from such an aggregate. The possibility that some solid fenoterol particles were associated with the lipid aggregates in Figure 3 could not be excluded. These might have acted as nuclei for the formation of lipid aggregates and hence functioned as reservoir of solid drug which would gradually have released the drug over a period of time. We believe that at the site of  
 15 application (in-vivo) the majority of particles initially deposit as PHA followed by a gradual rather than an instantaneous process of conversion into liposomes.

EXAMPLE VISpray Characteristics

For inhalation applications, it is desirable that the majority of the droplets should be predominantly in the 2 to 7 micron range. Only particles in this range are capable of reaching the lower regions of the lung. Measurements of the droplet size from two compositions with lipid/drug ratios 6:1 and 12:1 were carried out  
 25 in a Laser Beam Droplet & Particle Size Analyser (Malvern Instruments). Figures 4a and 4b show the effect of lipid/drug ratios on droplet size. The mean proportion of droplets of particle diameter below 7.5  $\mu$  is seen to decrease from a cumulative value of about 65% to 55% as the lipid/drug ratio increases from 6:1 (Figure 4a) to 12:1 (Figure 4b).

In order to determine the proportion of the drug dose delivered within the respirable range, measurements were made using a cascade impactor (C.F. Casell and Co., London). This type of cascade impactor  
 30 consists essentially of a system of four air-jets impinging in series on glass discs. The successive jets are progressively finer, so that the speed and, therefore, the efficiency of impaction of particles on to the discs increases from jet to jet, when air is drawn through at a steady rate. A size-grading thereby results which is used to assess the samples.

35 The drug collected on Plate 1 (particle range 7 - 20  $\mu\text{m}$  diameter) was assumed to be contained within droplets that would be too large to be inhaled. Droplets in this size range would normally be expected to impact on the throat and be ingested. Drug collected on Plate 4 (particle range up to 0.7  $\mu\text{m}$  diameter) was assumed to be contained in drops so small that they would probably be immediately exhaled. Only drug collected on Plates 2 and 3 (particle range 0.7 - 7.0  $\mu\text{m}$ ) was considered to lie within the respirable range.

40 A series of sprays (12:1 lipid/drug ratio) were prepared, which had vapour pressures ranging from 41 psi to 64 psi, the highest pressure at which samples could easily be prepared (by using different proportions of Arcton 11 and 12). These formulations were sprayed into the cascade impactor through which an air-flow of 17.5 litres per minute was being drawn. Each formulation was sprayed through a 0.75 mm nozzle and also through one with a finer orifice (0.5 mm). The amounts of drug impacting on Plates 3  
 45 and 4 were determined by HPLC. The results of these measurements are presented in Table II.

50

55

TABLE II

RESPIRABLE DOSE (ug) DELIVERED PER ACTUATION BY TYPE 1 (12:1) AEROSOLS FITTED WITH DIFFERENT NOZZLES MEASURED AT DIFFERENT PROPELLANT VAPOUR PRESSURES					
Sample	Vapour Pressure (psi)				
	41	47	51	55	60
Standard Nozzle (at least 0.75 mm)	12.4	8.7	14.9	10.6	13.1
Small Nozzle (0.5 mm)	22.6	16.4	20.7	23.7	25.8

They indicate that changes in vapour pressure have little effect on the fraction of the original dose delivered in the respirable range using the same nozzle size. Increasing the vapour pressure of the samples did, however, decrease the 7 - 20 micron component. The biggest improvement in drug delivery was attained using the smaller nozzle. This was evident at all the vapour pressures employed.

On the basis of these measurements, applicants are of the opinion that by selecting an appropriate combination of vapour pressure and nozzle size the delivery of pro-liposome droplets within the respirable range can be maximised.

#### EXAMPLE VII

##### In-vitro Sustained Release Comparisons

The sustained release properties of the compositions according to the invention have so far been demonstrated under in-vitro conditions. The data obtained provide good evidence that the present invention provides for the sustained release of a biologically active compound. It has also been demonstrated that the compositions of the invention are at least as effective as pro-liposome aerosol compositions disclosed in our earlier publication EP-A-0158441 in delaying the release of a biologically active compound. A composition according to the present invention, designated Type I, was tested against two other formulations (Type II and Type III) disclosed in publication EP-A-0158441.

TYPE I: A composition according to the present invention where the micronised drug is dispersed uniformly in a blend of Arcton 11, 12 and 114, having dissolved therein a lipid.

TYPE II: In this example, the biologically active compound was dissolved in a pro-liposome composition containing a small quantity of alcohol as solvent for the biologically active compound. The propellant was the same as in Type I.

TYPE III: In this example the drug was dissolved in a pro-liposome composition which contained both alcohol and water. The propellant was the same as in Types I and II.

Liposomal suspensions of all three formulations were formed in-situ by spraying into an aqueous reservoir as previously described.

In this experiment, the release of drug from the liposomal suspensions was determined in a diffusion cell. The preparation was placed on one side of a 4 cm diameter dialysis membrane in the cell. Buffer was pumped through a narrow space under this membrane at a fixed rate of 2 ml per minute. The solution emerging from the membrane cell was returned to the reservoir for recirculation. The rate of diffusion of the drug through the membrane was monitored by HPLC.

It can be seen from Figure 5 that all three pro-liposome aerosol compositions markedly delayed the release of fenoterol (compared to a control solution of the drug) over a period of 12 hours. Figure 5 shows sustained release profiles of sprayed liposome suspensions prepared from Type I, Type II and Type III aerosols. The aerosol preparations were sprayed into fixed volumes of water to form liposomes and collected as described above. 5 ml aliquots were then placed in a membrane cell for measurement of drug release. Each sample contained 5 mg ml<sup>-1</sup> fenoterol and 60 mg ml<sup>-1</sup> of lecithin giving a lipid/drug ratio of (12:1). It may, however, be noted that according to the in-vitro model, Type III > I > II in terms of sustained release profiles.

#### EXAMPLE VIII

##### In-vivo Sustained Release Comparisons

It was considered desirable that the in-vitro findings should be extrapolated to in-vivo situations. Therefore in order to establish in-vitro/in-vivo correlation, the following controlled studies were carried out on guinea pigs. Individual guinea pigs were exposed to a histamine aerosol in an inhalation chamber. After a short induction period, acute shortness of breath (dyspnea and tachypnea) was provoked, forcing the animal to lie on its side. If initial treatment with a bronchodilator (eg. a B<sub>2</sub> sympathomimetic) was administered prior to histamine challenge, the induction time before dyspnea set in may be delayed. The period of induction can therefore be used to evaluate the bronchodilatory effect of a biologically active compound or formulation. The method is based on data by P. Kallos and W. Pagel (Acta. Meda. Scand. 91 (1937) 292).

In an extension of this method, the time interval between initial treatment with a bronchodilator and challenge with histamine was progressively increased. The length of the induction period before dyspnea set in gave an indication of the duration of action of the bronchodilator. Therefore, the sustained release properties of various formulations could be further compared.

Test Procedure: The tests were carried out on male and female Pirbright white guinea pigs each weighing between 250 to 300 gm. Each animal was housed in a separate 2 l glass chamber during the test. At time zero, a fixed dose of aerosol containing fenoterol was sprayed into the chamber. In a parallel control, another guinea pig was not exposed to any bronchodilator formulation. After an interval of 120 minutes the two guinea pigs were each challenged with a standard dose of 0.2% histamine. The elapsed time in seconds between administration of 0.2% histamine and the first appearance of dyspnea was recorded. A fresh animal was used each time and each animal was only used once.

In a further series of experiments the time interval between pre-treatment with the bronchodilator sprays and challenge with histamine was increased to 180 minutes. A typical set of results from 2 formulations based on the invention is shown in Table III. It gives the induction period (in seconds) for individual guinea pigs after initial treatment with the spray material containing 400 ug fenoterol in a 6:1 (LH 18) and 12:1 (LH 19), lipid/drug ratio. For comparison, the results from six untreated guinea pigs (control) are shown.

TABLE III

ELAPSED TIME IN SECONDS BETWEEN EXPOSURE TO FENOTEROL AND ONSET OF DYSPNEA				
Control	After 120 Minutes		After 180 Minutes	
	LH18	LH19	LH18	LH19
84	298	185	138	98
86	601	215	140	158
58	163	138	210	88
88	171	140	208	164
66	185	201	72	118
86	191	208	91	300

Formulations based on variants of the invention (Type I) were compared against Type II and Type III formulations.

The ranking order of the various formulations based on the scores obtained are set out in Table IV. The statistical method for determining the ranking order of the different formulations was based on the Rang-Summon Test (ref: Lehrman, E.L. Non parametrics: Statistical Methods based on ranks, San Francisco, Holden-Day 1975).



TABLE IV

RANKING ORDER OF VARIOUS FORMULATIONS AFTER 120 MINUTES					
Formulation	Type	Fenoterol	Lipid/Drug	n	Sum of Scores
LH18	I	400 µg	6:1	6	233.5
LH19	I	400 µg	12:1	6	198.00
LH 1	I	200 µg	12:1	6	139.67
LH 4	II	400 µg	6:1	12	127.67
LH 7	III	400 µg	6:1	6	127.67
LH 9	II	400 µg	12:1	6	101.00
LH12	III	400 µg	12:1	6	86.71
CONTROL		NIL	NIL	30	41.18

It can be seen from Table IV that there was broad correlation between the in-vitro and in-vivo data. Type I, Type II and Type III formulations all had sustained release properties compared with a control after 120 minutes.

The prolonged effect of the Type I compositions was particularly evident when the interval between initial treatment and subsequent challenge with histamine was increased to 180 minutes. It would appear that after 180 minutes fenoterol protected in the pro-liposome droplet of the invention was twice as active as the unprotected drug. This is shown in Table V.

TABLE V

RANKING ORDER OF THE INVENTION WITH 12:1; 6:1; LIPID/DRUG RATIOS AFTER 180 MINUTES					
Formulation	Type	Fenoterol	Lipid/Drug	n	Sum of Scores
LH18	I	400 µg	6:1	6	208.5
LH19	I	400 µg	12:1	6	190.5
UNPROTECTED DRUG				6	106.00
CONTROL				6	43.00

It also appears that with this invention a lipid/drug ratio of 6:1 is as effective as 12:1 in providing sustained bronchodilatory effect after 180 minutes in experimental animals.

The following examples demonstrate the high initial entrapment and improved retention of powder compositions according to the present invention.

#### EXAMPLE IX

50 mg of micronised fenoterol HBr was dispersed uniformly in a solution of soy-bean lecithin (600 mg) 95% pure in 2 g of Arcton 11. The Arcton 11 was allowed to flash off, leaving behind a dispersion of fenoterol particles in lipid. To this dispersion, 5 g of micronised lactose was added. The resultant powder composition was tumbled in a ball-mill to ensure uniform mixing.

#### EXAMPLE X

In a variation of this method, the lactose was added to the suspension of fenoterol in Arcton 11 with the lipid in solution. The Arcton 11 was flashed off, leaving behind a uniform powder composition.

In both examples IX and X, the fenoterol was in solid dispersion in the lipid.

#### EXAMPLE XI (Comparative)

In this example, 50 mg fenoterol was first dissolved in 2 g of ethanol. 600 mg of lipid was solvated in 4 g of Arcton 11. The 2 solutions were combined and loaded gradually onto 5 g of lactose. The solvents were evaporated off in a stream of nitrogen to leave behind a powder composition wherein the drug was in

solution in the lipid. This example produced a powder composition that resembled the one described by Payne insofar as that the drug was in solution in the lipid.

#### EXAMPLE XII

5

The three components were separately added to a ball-mill or air jet microniser to obtain the required particle size and dispersion of drug in lipid. In this case, no solvents were required.

The measurements for initial entrapment and in-vitro release of fenoterol from the four examples IX-XII were carried out in similar fashion to the aerosol compositions. Figure 6 shows the release of fenoterol as a function of time for the four formulations:

- Drug in suspension, and lipid dissolved, in Arcton 11. Lactose added, solvents removed by rotary evaporation.
- Drug and lipid in solution in 2:1 Arcton 11/ethanol (V:V) mixture. Lactose added, solvents removed by rotary evaporation.
- ◇ Drug in suspension in Arcton 11 containing lipid. Arcton 11 evaporated prior to lactose addition.
- △ Lactose, lipid, drug milled in tumble mill with no solvent addition.

It can be seen that Examples IX, X and XII which have the drug in suspension in the lipid show much higher initial entrapment than the formulation of Example XI. They also retained the drug much better than the formulation of Example XI, where the drug was in solution in the lipid. The reverse curvature of Example XI is believed to indicate a different mechanism of release of the fenoterol which becomes rapidly released into the aqueous phase as the lactose dissolves and then returns by partition into the lipid phase.

Drug entrapment and release profiles of the powder compositions according to the invention may be affected by lipid/drug ratios in like manner to the aerosol compositions previously described. Alternative lipid materials such as hydrogenated lipids may be used in place of soy-bean lecithin without interfering with the sustained release properties of the resulting composition. The compositions of Examples IX and X may be micronised further to obtain an average particle size, ideally between 0.5 and 8 microns, for inhalation applications. Similar release profiles were found for these further micronised compositions.

In all instances the average particle size of the final powder composition should be less than 100  $\mu$  and preferably below 60  $\mu$ .

30

#### EXAMPLE XIII

A spray formulation was sprayed through a nozzle to give a hydrogenated lipid of high melting point without hydrophilic carrier by forming micronised droplets of the drug in suspension in the lipid from which carrier has volatilised. The resulting material was collected.

Of course liposomes may also be formed by adding an excess of water to the pro-liposome powder composition comprising carrier, lipid and drug, the composition being free from other solvent for the lipid. In that case, the powder is simply contacted with excess water without first being dispersed in air. The invention therefore provides a pro-liposome composition wherein a membrane lipid and a particulate biologically active compound are minor components of discrete micronised particles whose major component is a physiologically acceptable solid carrier. The invention also includes making liposomes from a composition as aforesaid by contact with water either in situ or in-vivo. For oral administration the discrete micronised solid particles may be filled into gelatin or other capsules.

The compositions of the invention are suitable for the administration to a human or animal subject of an aerosol composition as aforesaid for the treatment of bronchial, pulmonary and nasal disorders such as asthma, bronchitis, hay fever and the like containing bronchodilators and steroids, antibiotics, anti-histamines or vasoconstrictors. They are also suitable for the administration of topical preparations where sustained release of the active compound or compounds is desirable, for example in the treatment of psoriasis and inflammatory skin conditions such as eczema with steroids.

50

#### Claims

1. A composition comprising a membrane lipid together with a biologically active compound and which has the property of spontaneously forming vesicles on contact with an excess of water, characterised in that:
  - (a) the composition is a solid which comprises discrete micronised particles;
  - (b) the biologically active compound is present in the form of discrete micronised particles; and
  - (c) the composition is free from solvent for the biologically active compound.

55

2. A composition according to claim 1, wherein the membrane lipid is a natural or hydrogenated lecithin, a glycolipid, a long chain dialkyl ammonium compound or a mixture of the aforesaid compounds with a compatible lipophilic material.
- 5 3. A composition according to claim 1 or 2, wherein the biologically active compound has the property of being released in a sustained manner.
4. A composition according to claim 3, wherein the biologically active compound is a bronchodilator, steroid, antibiotic, antihistamine, vasoconstrictor or anti-inflammatory compound.
- 10 5. A composition according to claim 2, wherein the biologically active compound is salbutamol, terbutaline, orciprenaline, isoprenaline, reproterol, pirbuterol, butenoside, beclamethasone dipropionate, sodium chromoglycate, fenoterol, ipratropium, betamethasone valerate, rimiterol or ketotifen.
- 15 6. A composition according to any preceding claim, wherein the biologically active compound is present as particles of average size from 0.5 to 8 micrometers.
7. The composition of any preceding claim, comprising particles in which the biologically active compound is in particulate dispersion in the lipid.
- 20 8. The composition of claim 7, wherein the composition has as a minor component particles of a biologically active compound in particulate dispersion in the lipid and as its major component particles of a physiologically acceptable solid carrier.
- 25 9. A composition according to claim 8, wherein the solid carrier is glucose, lactose, a dextran or starch.
10. A composition according to claim 9, wherein the weight ratio of membrane lipid to carrier is from 1:1 to 1:25.
- 30 11. A composition according to claim 10, wherein the weight ratio of biologically active compound to lipid is from 1:0.5 to 1:25.
12. A composition according to claim 11, wherein the weight ratio of biologically active compound to lipid is from 1:6 to 1:20.
- 35 13. A composition as claimed in any of claims 9 to 12, wherein the micronised solid particles of biologically active compound in particulate dispersion in the lipid are of average diameter 0.5 to 8 micrometers.
14. A composition according to any of claims 1 to 13, in which the particles are in dispersion in airstream
- 40 15. A capsule filled with a composition as claimed in any of claims 1 to 13.
16. A sprayable composition comprising at least one volatile liquid propellant, at least one bilayer lipid that is at least partially dissolved or dispersed in the propellant and at least one biologically active compound, characterised in that the biologically active compound is present in the propellant and/or the lipid as a discrete dispersed micronised powder, that the composition is free from solvent for the biologically active compound, and in that the composition has the property after it has been sprayed through a nozzle to form an aerosol of spontaneously forming vesicles on contact with an excess of water.
- 45 17. A composition according to claim 16, wherein the propellant used is a perhalocarbon.
18. A composition according to claim 17, wherein the propellant is  $\text{CClF}_3$ ,  $\text{CCl}_2\text{F}_2$  or  $\text{C}_2\text{Cl}_2\text{F}_4$ .
- 50 19. A composition according to any of claims 16 to 18, wherein the membrane lipid is a natural or hydrogenated lecithin, a glycolipid, a long chain dialkyl ammonium compound or a mixture of any of the aforesaid compounds with a compatible lipophilic material.
- 55

20. A composition according to any of claims 16 to 19, wherein the biologically active compound ionises and has an electrical charge that is of opposite electrical sign to the charge on the lipid material.
21. A composition according to claim 20, wherein an amphipath is present to confer charge on the lipid material.
22. A composition according to any of claims 16 to 21, wherein the biologically active compound is present as particles of average size from 0.5 to 8 micrometers.
23. A composition according to any of claims 16 to 22, wherein the biologically active compound is salbutamol, terbutaline, orciprenaline, isoprenaline, reproterol, pirbuterol, butenoside, beclamethasone dipropionate, sodium chromoglycate, fenoterol, ipratropium, betamethasone valerate, rimiterol or ketotifen.
24. A composition according to any of claims 16 to 23, wherein the biologically active compound and lipid are present in a weight ratio of at least 1:0.5.
25. A composition according to any of claims 16 to 24, wherein the biologically active compound and lipid are present in a weight ratio of from 1:3 to 1:12.
26. An aerosol canister charged with a single sprayable component that is a composition as claimed in any of claims 16 to 25.
27. A method for the preparation of pro-liposomes ("liposomes" being lipid bilayers surrounding an aqueous space and including niosomes and vesicles generally) which comprises forming discrete particles of at least one membrane lipid and at least one biologically active compound, such particles being free from solvent for the biologically active compound and said biologically active compound being present as discrete micronised particles.
28. A method according to claim 27, which comprises spraying under pressure through nozzle means a single composition comprising at least one volatile liquid propellant, at least one membrane lipid that is at least partly dissolved or dispersed in the propellant and at least one biologically active compound that is present in dispersion in the propellant and/or the lipid, the composition being free from solvent for the biologically active compound.
29. The method of claim 28, wherein the propellant used is a perhalocarbon.
30. The method of claim 29, wherein the propellant is  $\text{CClF}_3$ ,  $\text{CCl}_2\text{F}_2$  or  $\text{C}_2\text{Cl}_2\text{F}_4$ .
31. The method of any of claims 27 to 30, wherein the membrane lipid is a natural or hydrogenated lecithin, a glycolipid, a long chain dialkyl ammonium compound or a mixture of any of the aforesaid compounds with a compatible lipophilic material.
32. A method according to any of claims 27 to 31, wherein the biologically active compound is ionised and has an electrical charge that is of opposite electrical sign to the charge on the lipid material.
33. A method according to claim 32, wherein an amphipath is present to confer charge on the lipid material.
34. A method according to any of claims 27 to 33, wherein the biologically active compound is present as particles of average size from 0.5 to 8 micrometers.
35. A method according to any of claims 27 to 34, wherein the biologically active compound is salbutamol, terbutaline, orciprenaline, isoprenaline, reproterol, pirbuterol, butenoside, beclamethasone dipropionate, sodium chromoglycate, fenoterol, ipratropium, betamethasone valerate, rimiterol or ketotifen.
36. A method according to any of claims 27 to 35, wherein the biologically active compound and lipid are present in a weight ratio of at least 1:0.5.

37. A method according to claim 27, wherein the bilayer lipid and the biologically active compound are minor components of micronised solid particles whose major component is a physiologically acceptable solid carrier.
- 5 38. A method according to claims 37, wherein the solid carrier is glucose or lactose.
39. A method according to claim for 37 or 38, wherein the ratio of membrane lipid to carrier is from 1:1 to 1:25.
- 10 40. A method according to any of claims 37 to 39, wherein the weight ratio of biologically active compound to lipid is from 1:0.5 to 1:25.
41. A method according to any of claims 37 to 39, wherein the weight ratio of biologically active compound to lipid is from 1:6 to 1:20.
- 15 42. A method according to any of claims 37 to 47, comprising the further step of forming an aerosol of the particles by shaking the particles into a moving airstream or by introducing the particles from a container into a moving airstream.
- 20 43. A method according to claim 42, wherein the micronised solid particles of pro-liposome are of average diameter 0.5 to 8 micrometers.
44. Use for the formation of liposomes or niosomes of a pharmaceutical composition comprising a membrane lipid together with a biologically active compound and which has the property of spontaneously forming vesicles on contact with an excess of water, characterised in that:
- 25 (a) the composition is a solid which comprises discrete micronised particles;  
(b) the biologically active compound is present in the form of discrete micronised particles; and  
(c) the composition is free from solvent for the biologically active compound,
- 30 the resulting vesicles exhibiting high initial entrapment of the biologically active compound together with sustained release of the biologically active compound at the site of application.

#### Patentansprüche

- 35 1. Zusammensetzung bestehend aus einem Membranlipid und einer biologisch aktiven Verbindung, wobei es beim Kontakt mit Überschußwasser spontan zur Vesikelbildung kommt, dadurch gekennzeichnet, daß:
- (a) die Zusammensetzung ein Feststoff bestehend aus diskreten, mikronisierten Partikeln ist;  
(b) die biologisch aktive Verbindung in Form von diskreten, mikronisierten Partikeln vorhanden ist;  
40 und  
(c) die Zusammensetzung kein Lösungsmittel für die biologisch aktive Verbindung aufweist.
2. Zusammensetzung nach Anspruch 1, wobei das Membranlipid ein natürliches oder hydriertes Lecithin, ein Glykolipid, oder eine langkettige Dialkyl-Ammonium-Verbindung oder eine Mischung der zuvor genannten Verbindungen zusammen mit einer kompatiblen fettfreundlichen Substanz ist.
- 45 3. Zusammensetzung nach Anspruch 1 oder 2, wobei die biologisch aktive Verbindung die Eigenschaft hat, nachhaltig zu wirken.
- 50 4. Zusammensetzung nach Anspruch 3, wobei die biologisch aktive Verbindung ein Bronchodilatator, Steroid, Antibiotikum, Antihistaminikum, Vasokonstriktor oder eine antiinflammatorische Verbindung ist.
5. Zusammensetzung nach Anspruch 2, wobei die biologisch aktive Verbindung Salbutamol, Terbutalin, Orciprenaline, Isoprenaline, Reproterol, Pirbuterol, Butenosid, Beclamethason-Dipropionat, Natrium-Chromoglycat, Fenoterol, Ipratropium, Beta-Methason-Valerat, Rimiterol oder Ketotifen ist.
- 55 6. Zusammensetzung nach einem der vorhergehenden Ansprüche, wobei die biologisch aktive Verbindung als Partikel mit einer Durchschnittsgröße von 0,5 bis 8 Mikrometern vorhanden ist.

7. Zusammensetzung nach einem der vorhergehenden Ansprüche bestehend aus Partikeln, wobei die biologisch aktive Verbindung partikulär im Lipid dispergiert ist.
8. Zusammensetzung nach Anspruch 7, wobei die Partikel der partikulär im Lipid dispergierten biologisch aktiven Verbindung den geringeren Teil und Partikel einer physiologisch akzeptablen Festträgersubstanz den größeren Teil der Zusammensetzung bilden.
9. Zusammensetzung nach Anspruch 8, wobei die Festträgersubstanz Glukose, Lactose, ein Dextran oder Stärke ist.
10. Zusammensetzung nach Anspruch 9 mit einem Gewichtsverhältnis zwischen dem Membranlipid und der Trägersubstanz von 1:1 bis 1:25.
11. Zusammensetzung nach Anspruch 10 mit einem Gewichtsverhältnis zwischen der biologisch aktiven Verbindung und dem Lipid von 1:0,5 bis 1:25.
12. Zusammensetzung nach Anspruch 11 mit einem Gewichtsverhältnis zwischen der biologisch aktiven Verbindung und dem Lipid von 1:6 bis 1:20.
13. Zusammensetzung nach einem der Ansprüche 9 bis 12, wobei die mikronisierten Festpartikel der biologisch aktiven Verbindung partikulär im Lipid dispergiert sind und einen Durchmesser von 0,5 bis 8 Mikrometern aufweisen.
14. Zusammensetzung nach einem der Ansprüche 1 bis 13, wobei die Partikel im Luftstrom dispergiert sind.
15. Eine Kapsel, gefüllt mit einer Zusammensetzung nach einem der Ansprüche 1 bis 13.
16. Sprühbare Zusammensetzung bestehend aus wenigstens einer flüchtigen Flüssigtreibgas Mischung, wenigstens einer Lipiddoppelschicht, die wenigstens teilweise in der Treibgas Mischung aufgelöst oder dispergiert ist, und wenigstens einer biologisch aktiven Verbindung, dadurch gekennzeichnet, daß die biologisch aktive Verbindung in der Treibgas Mischung und/oder dem Lipid als diskretes, dispergiertes und mikronisiertes Pulver vorhanden ist, daß die Zusammensetzung frei von Lösungsmitteln für die biologisch aktive Zusammensetzung ist und daß die Zusammensetzung, nachdem sie durch eine Düse gesprüht wurde, ein Aerosol bildet, wobei es beim Kontakt mit Überschußwasser zur spontanen Bildung von Vesikeln kommt.
17. Zusammensetzung nach Anspruch 16, wobei die verwendete Treibgas Mischung ein Perhalo-Kohlenstoff ist.
18. Zusammensetzung nach Anspruch 17, wobei die Treibgas Mischung ein  $\text{CClF}_3$ ,  $\text{CCl}_2\text{F}_2$  oder  $\text{C}_2\text{Cl}_2\text{F}_4$  ist.
19. Zusammensetzung nach einem der Ansprüche 16 bis 18, wobei das Membranlipid ein natürliches oder hydriertes Lecithin, ein Glykolipid, eine langkettige Dialkyl-Ammonium-Verbindung oder eine beliebige Mischung der zuvor genannten Verbindungen zusammen mit einer kompatiblen fettfreundlichen Substanz ist.
20. Zusammensetzung nach einem der Ansprüche 16 bis 19, wobei die biologisch aktive Verbindung zu Ionen zerfällt und zur Lipidsubstanz eine entgegengesetzte elektrische Ladung aufweist.
21. Zusammensetzung nach Anspruch 20, wobei ein Amphipath zum Übertragen der Ladung in die Lipidsubstanz vorhanden ist.
22. Zusammensetzung nach einem der Ansprüche 16 bis 21, wobei die biologisch aktive Verbindung als Partikel mit einer Durchschnittsgröße von 0,5 bis 8 Mikrometern vorhanden ist.
23. Zusammensetzung nach einem der Ansprüche 16 bis 22, wobei die biologisch aktive Substanz

Salbutamol, Terbutalin, Orciprenalin, Isoprenalin, Reproterol, Pirbuterol, Butenosid, Beclamethason-Dipropionat, Natrium-Chromoglycat, Fenoterol, Ipratropium, Beta-Methason-Valerat, Rimiterol oder Ketotifen ist.

- 5 24. Zusammensetzung nach einem der Ansprüche 16 bis 23, wobei die biologisch aktive Verbindung und das Lipid ein Gewichtsverhältnis von wenigstens 1:0,5 aufweisen.
25. Zusammensetzung nach einem der Ansprüche 16 bis 24 mit einem Gewichtsverhältnis zwischen der biologisch aktiven Verbindung und dem Lipid von 1:3 bis 1:12.
- 10 26. Aerosolbehälter, gefüllt mit einer einzelnen sprühbaren Komponente bestehend aus einer Zusammensetzung nach einem der Ansprüche 16 bis 25.
27. Verfahren zur Herstellung von Proliposomen (wobei mit "Liposomen" lipide Doppelschichten, einschließlich Niosome und Vesikel im allgemeinen, bezeichnet werden, die einen wässrigen Raum umschließen), wobei diskrete Partikel aus wenigstens einem Membranlipid und wenigstens einer biologisch aktiven Verbindung gebildet werden und diese Partikel frei von Lösungsmitteln für die biologisch aktiven Verbindung sind und die genannte biologisch aktive Verbindung in Form von diskreten, mikronisierten Partikeln vorhanden ist.
- 15 28. Verfahren nach Anspruch 27, wobei eine einzelne Zusammensetzung unter Druck durch eine Düse gesprüht wird, bestehend aus wenigstens einer flüchtigen Flüssigtreibgas Mischung, wenigstens einem Membranlipid, das in der Treibgas Mischung wenigstens teilweise aufgelöst oder dispergiert ist und wenigstens einer biologisch aktiven Verbindung, die in der Treibgas Mischung und/oder dem Lipid dispergiert vorhanden ist, wobei die Zusammensetzung frei von Lösungsmitteln für die biologisch aktive Verbindung ist.
- 20 29. Verfahren nach Anspruch 28, wobei die Treibgas Mischung ein Perhalo-Kohlenstoff ist.
- 30 30. Verfahren nach Anspruch 29, wobei die Treibgas Mischung ein  $\text{CClF}_3$ ,  $\text{CCl}_2\text{F}_2$  oder  $\text{C}_2\text{Cl}_2\text{F}_4$  ist.
31. Verfahren nach einem der Ansprüche 27 bis 30, wobei das Membranlipid ein natürliches oder hydriertes Lecithin, ein Glykolipid, eine langkettige Dialkyl-Ammonium-Verbindung oder eine beliebige Mischung der zuvor genannten Verbindungen zusammen mit einer kompatiblen fettfreundlichen Substanz ist.
- 35 32. Verfahren nach einem der Ansprüche 27 bis 31, wobei die biologisch aktive Verbindung zu Ionen zerfällt und zur Lipidsubstanz eine entgegengesetzte elektrische Ladung aufweist.
- 40 33. Verfahren nach Anspruch 32, wobei ein Amphipath zum Übertragen der Ladung in die Lipidsubstanz vorhanden ist.
34. Verfahren nach einem der Ansprüche 26 bis 33, wobei die biologisch aktive Verbindung als Partikel mit einer Durchschnittsgröße von 0,5 bis 8 Mikrometern vorhanden ist.
- 45 35. Verfahren nach einem der Ansprüche 27 bis 34, wobei die biologisch aktive Verbindung Salbutamol, Terbutalin, Orciprenalin, Isoprenalin, Reproterol, Pirbuterol, Butenosid, Beclamethason-Dipropionat, Natrium-Chromoglycat, Fenoterol, Ipratropium, Beta-Methason-Valerat, Rimiterol oder Ketotifen ist.
- 50 36. Verfahren nach einem der Ansprüche 27 bis 35, wobei die biologisch aktive Verbindung und das Lipid ein Gewichtsverhältnis von wenigstens 1:0,5 aufweisen.
37. Verfahren nach Anspruch 27, wobei die Lipiddoppelschicht und die biologisch aktive Verbindung als mikronisierte Festpartikel den geringeren Teil und eine physiologisch akzeptable Festträgersubstanz den größeren Teil ausmacht.
- 55 38. Verfahren nach Anspruch 37, wobei die Trägersubstanz Glukose oder Lactose ist.

39. Verfahren nach Anspruch 37 oder 38, wobei das Gewichtsverhältnis zwischen Membranlipid und Trägersubstanz 1:1 bis 1:25 beträgt.
40. Verfahren nach einem der Ansprüche 37 bis 39, wobei das Gewichtsverhältnis zwischen der biologisch aktiven Verbindung und dem Lipid 1:0,5 bis 1:25 beträgt.
41. Verfahren nach einem der Ansprüche 37 bis 39, wobei das Gewichtsverhältnis zwischen der biologisch aktiven Substanz und dem Lipid 1:6 bis 1:20 beträgt.
42. Verfahren nach einem der Ansprüche 37 bis 41, umfassend den weiteren Schritt der Bildung eines Aersols von Partikeln durch Schütteln der Partikel in einem bewegten Luftstrom oder durch Einführen der Partikel aus einem Behälter in einen bewegten Luftstrom.
43. Verfahren nach Anspruch 42, wobei die mikronisierten Proliposom-Festpartikel einen durchschnittlichen Durchmesser von 0,5 bis 8 Mikrometer haben.
44. Verwendung einer pharmazeutischen Zusammensetzung bestehend aus einem Membranlipid und einer biologisch aktiven Verbindung für die Bildung von Liposomen oder Niosomen, wobei es in Verbindung mit Überschußwasser spontan zur Vesikelbildung kommt, dadurch gekennzeichnet, daß:
- (a) die Zusammensetzung ein Feststoff bestehend aus diskreten, mikronisierten Partikeln ist;
  - (b) die biologisch aktive Verbindung in Form von diskreten, mikronisierten Partikeln vorhanden ist; und
  - (c) die Zusammensetzung frei von Lösungsmitteln für die biologisch aktive Verbindung ist,
- wobei in den resultierenden Vesikeln eine anfänglich hohe Einschlußmenge an biologisch aktiver Verbindung vorhanden ist und die biologisch aktive Verbindung eine nachhaltige Wirkung am Verabreichungsort aufweist.

#### Revendications

1. Composition comprenant un lipide membraneux et un composé biologiquement actif et qui a la propriété de former spontanément des vésicules au contact avec un excès d'eau: caractérisée en ce que :
- (a) la composition est un solide qui comprend des particules micronisées discrètes ;
  - (b) le composé biologiquement actif est présent sous forme de particules micronisées discrètes ; et
  - (c) la composition est libre de solvant pour le composé biologiquement actif.
2. Composition qui, selon la revendication 1, est caractérisée en ce que la membrane lipidique est une lécithine naturelle ou hydrogénée, un glucolipide, un composé d'ammonium de dialcyle à longue chaîne ou un mélange desdits composés ci-dessus avec une matière lipophile compatible.
3. Composition qui, selon les revendications 1 ou 2, est caractérisée en ce que le composé biologiquement actif a la propriété d'être libéré d'une manière soutenue.
4. Composition qui, selon la revendication 3, est caractérisée en ce que le composé biologiquement actif est un bronchodilatateur, un stéroïde, un antibiotique, un antihistaminique, un vasoconstricteur ou un composé anti-inflammatoire.
5. Composition qui, selon la revendication 2, est caractérisée en ce que le composé biologiquement actif consiste en salbutamol, terbutaline, orciprénaline, isoprénaline, réprotérol, pirbutérol, buténoside, bécclaméthasone dipropionate, chromoglycate de sodium, fénotérol, ipratropium, bétaméthasone-valérate, rimitérol ou kétotifène.
6. Composition qui, selon toute revendication précédente, est caractérisée en ce que le composé biologiquement actif est présent sous forme de particules d'une taille moyenne de 0,5 à 8 micromètres.
7. Composition conforme à toute revendication précédente, comprenant des particules dans lesquelles le composé biologiquement actif est en dispersion particulaire dans le lipide.



8. Composition conforme à la revendication 7, caractérisée en ce que la composition possède comme constituant mineur des particules d'un composé biologiquement actif en dispersion particulaire dans le lipide et comme constituant majeur des particules d'un véhicule solide physiologiquement acceptable.
- 5 9. Composition qui, selon la revendication 8, est caractérisée en ce que le véhicule solide consiste en glucose, en lactose, en dextran ou en amidon.
- 10 10. Composition qui, selon la revendication 9, est caractérisée en ce que le rapport pondéré entre le lipide membraneux et le véhicule est de 1:1 à 1:25.
- 10 11. Composition qui, selon la revendication 10, est caractérisée en ce que le rapport pondéré entre le composé biologiquement actif et le lipide est de 1:0,5 à 1:25.
- 15 12. Composition qui, selon la revendication 11, est caractérisée en ce que le rapport pondéré entre le composé biologiquement actif et le lipide est de 1:6 à 1:20.
- 20 13. Composition qui, selon l'une quelconque des revendications de 9 à 12, est caractérisée en ce que les particules solides micronisées de composé biologiquement actif en dispersion particulaire dans le lipide sont d'un diamètre moyen de 0,5 à 8 micromètres.
- 20 14. Composition qui, selon l'une quelconque des revendications de 1 à 13, est caractérisée en ce que les particules sont en dispersion dans un jet d'air.
- 25 15. Capsule remplie d'une composition selon la revendication d'une quelconque des revendications de 1 à 13.
- 30 16. Composition pulvérisable comprenant au moins un pulseur volatil liquide, au moins un lipide de doubles couches qui est au moins partiellement dissout ou dispersé dans le pulseur et au moins un composé biologiquement actif, caractérisée en ce que le composé biologiquement actif est présent dans le pulseur et/ou le lipide en tant que poudre micronisée dispersée discrète, que la composition est libre de solvant pour le composé biologiquement actif, et que la composition a la propriété, après avoir été pulvérisée au moyen d'un ajutage de former un aérosol formant spontanément des vésicules au contact avec un excès d'eau.
- 35 17. Composition qui, selon la revendication 16, est caractérisée en ce que le pulseur utilisé consiste en perhalocarbone.
- 40 18. Composition qui, selon la revendication 17, est caractérisée en ce que le pulseur consiste en  $\text{CClF}_3$ , en  $\text{CCl}_2\text{F}_2$  ou en  $\text{C}_2\text{Cl}_2\text{F}_4$ .
- 45 19. Composition qui, selon l'une quelconque des revendications de 16 à 18, est caractérisée en ce que le lipide membraneux est une lécithine naturelle ou hydrogénée, un glucolipide, un composé à base d'ammonium dialkyle à longue chaîne au un mélange de l'un desdits composés ci-dessus avec une matière lipophile compatible.
- 50 20. Composition qui, selon l'une quelconque des revendications de 16 à 19, est caractérisée en ce que le composé biologiquement actif est ionisé et a une charge électrique dont la polarité est opposée à la charge de la matière lipidique.
- 55 21. Composition qui, selon la revendication 20, est caractérisée en ce qu'un véhicule amphipathique est présent pour transmettre une charge à la matière lipidique.
22. Composition qui, selon l'une quelconque des revendications de 16 à 21, est caractérisée en ce que le composé biologiquement actif est présent sous forme de particules dont la taille moyenne est de 0,5 à 8 micromètres.
23. Composition qui, selon l'une quelconque des revendications de 16 à 22, est caractérisée en ce que le composé biologiquement actif consiste en salbutamol, terbutaline, orciprénaline, isoprénaline, réproté-

rol, pirbutérol, buténoside, béclaméthasone dipropionate, chromoglycate de sodium, fénotérol, ipratropium, bétaméthasone-valérate, rimitérol ou kétotifène.

24. Composition qui, selon l'une quelconque des revendications de 16 à 23, est caractérisée en ce que le  
5 composé biologiquement actif et le lipide sont présents sous un rapport pondéré d'au moins 1:0,5.
25. Composition qui, selon l'une quelconque des revendications de 16 à 24, est caractérisée en ce que le  
composé biologiquement actif et le lipide sont présents sous un rapport pondéré allant de 1:3 à 1:12.
- 10 26. Atomiseur d'aérosol chargé d'un seul composant pulvérisable qui consiste en une composition  
revendiquée dans l'une des revendications de 16 à 25.
27. Méthode pour la préparation de pro-liposomes (les "liposomes" étant des doubles couches lipidiques  
15 entourant un espace aqueux et comprenant des niosomes et des vésicules en règle générale) qui  
comprend la formation de particules discrètes d'au moins un lipide membraneux et au moins un  
composé biologiquement actif, ces particules étant libres de solvant pour le composé biologiquement  
actif et ledit composé biologiquement actif étant présent en tant que particules micronisées discrètes.
28. Méthode qui, selon la revendication 27, comprend la pulvérisation sous pression au moyen d'un ajutage  
20 d'une composition unique comprenant au moins un pulseur volatil liquide, au moins un lipide  
membraneux qui est au moins partiellement dissout ou dispersé dans le pulseur et au moins un  
composé biologiquement actif qui est présent en dispersion dans le pulseur et/ou le lipide, la  
composition étant libre de solvant pour le composé biologiquement actif.
- 25 29. La méthode de la revendication 28 qui est caractérisée en ce que le pulseur utilisé consiste en  
perhalocarbone.
30. La méthode de la revendication 29 qui est caractérisée en ce que le pulseur consiste en  $\text{CClF}_3$ , en  
 $\text{CCl}_2\text{F}_2$  ou en  $\text{C}_2\text{Cl}_2\text{F}_4$ .  
30
31. La méthode de l'une des revendications de 27 à 30, caractérisée en ce que le lipide membraneux est  
une lécithine naturelle ou hydrogénée, un glucolipide, un composé à base d'ammonium dialkyle à  
longue chaîne ou un mélange de l'un quelconque desdits composés ci-dessus avec une matière  
lipophile compatible.  
35
32. Méthode qui, selon l'une quelconque des revendications de 27 à 31, est caractérisée en ce que le  
composé biologiquement actif est ionisé et a une charge électrique dont la polarité est opposée à la  
charge de la matière lipidique.
- 40 33. Méthode qui, selon la revendication 32, est caractérisée en ce qu'un véhicule amphipathique est  
présent pour transmettre la charge à la matière lipidique.
34. Méthode qui, selon l'une quelconque des revendications de 27 à 33, est caractérisée en ce que le  
composé biologiquement actif est présent en tant que particules dont la taille moyenne est de 0,5 à 8  
45 micromètres.
35. Méthode qui, selon l'une quelconque des revendications de 27 à 34, est caractérisée en ce que le  
composé biologiquement actif consiste en salbutamol, terbutaline, orciprénaline, isoprénaline, réproté-  
rol, pirbutérol, buténoside, béclaméthasone dipropionate, chromoglycate de sodium, fénotérol, ipratro-  
pium, bétaméthasone-valérate, rimitérol ou kétotifène.  
50
36. Méthode qui, selon l'une quelconque des revendications de 27 à 35, est caractérisée en ce que le  
composé biologiquement actif et le lipide sont présents sous un rapport pondéré d'au moins 1:0,5.
- 55 37. Méthode qui, selon la revendication 27, est caractérisée en ce que le lipide des doubles couches et le  
composé biologiquement actif sont des constituants mineurs de particules solides micronisées dont le  
constituant majeur est un véhicule solide physiologiquement acceptable.

38. Méthode qui, selon la revendication 37, est caractérisée en ce que le véhicule solide consiste en  
glucose ou en la lactose.
39. Méthode qui, selon les revendications 37 ou 38, est caractérisée en ce que le rapport entre le lipide  
5 membraneux et le véhicule est de 1:1 à 1:25.
40. Méthode qui, selon l'une quelconque des revendications de 37 à 39, est caractérisée en ce que le  
rapport pondéré entre le composé biologiquement actif et le lipide est de 1:0,5 à 1:25.
- 10 41. Méthode qui, selon l'une quelconque des revendications de 37 à 39, est caractérisée en ce que le  
rapport pondéré entre le composé biologiquement actif et le lipide est de 1:6 à 1:20.
42. Méthode qui, selon l'une quelconque des revendications de 37 à 41, comprend la manière additionnelle  
15 de former un aérosol de particules en agitant les particules dans un jet d'air ou en faisant passer les  
particules d'un récipient dans un jet d'air mobile.
43. Méthode qui, selon la revendication 42, est caractérisée en ce que les particules solides micronisées  
de pro-liposomes ont un diamètre moyen de 0,5 à 8 micromètres.
- 20 44. Utilisation pour la formation des liposomes ou des niosomes d'une composition pharmaceutique  
comprenant un lipide membraneux et un composé biologiquement actif et qui a la propriété de former  
spontanément des vésicules au contact avec un excès d'eau, caractérisée en ce que:
- a) la composition est un solide qui contient des particules micronisées discrètes ;  
b) le composé biologiquement actif est présent sous forme de particules micronisées discrètes ; et  
25 c) la composition est libre de solvant pour le composé biologiquement actif,  
et que les vésicules en résultant accusent un niveau initial élevé de prise au piège du composé  
biologiquement actif ainsi qu'une libération soutenue du composé biologiquement actif au site  
d'application.

30

35

40

45

50

55

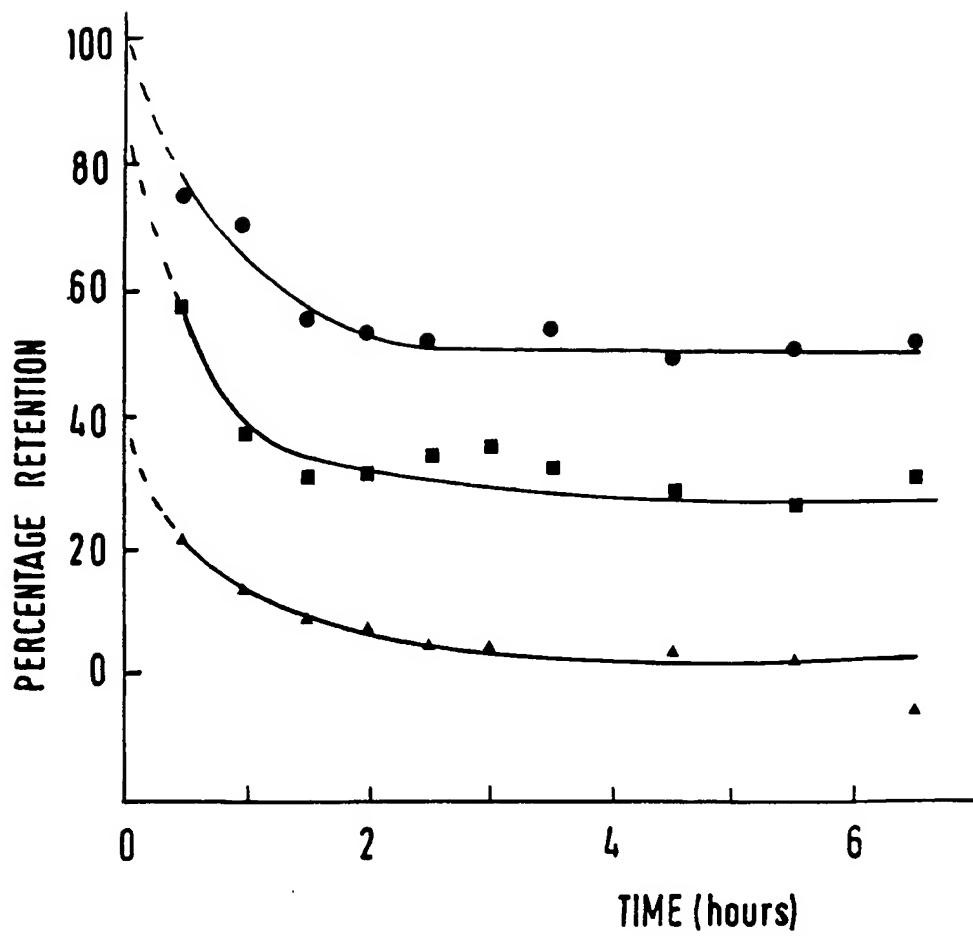
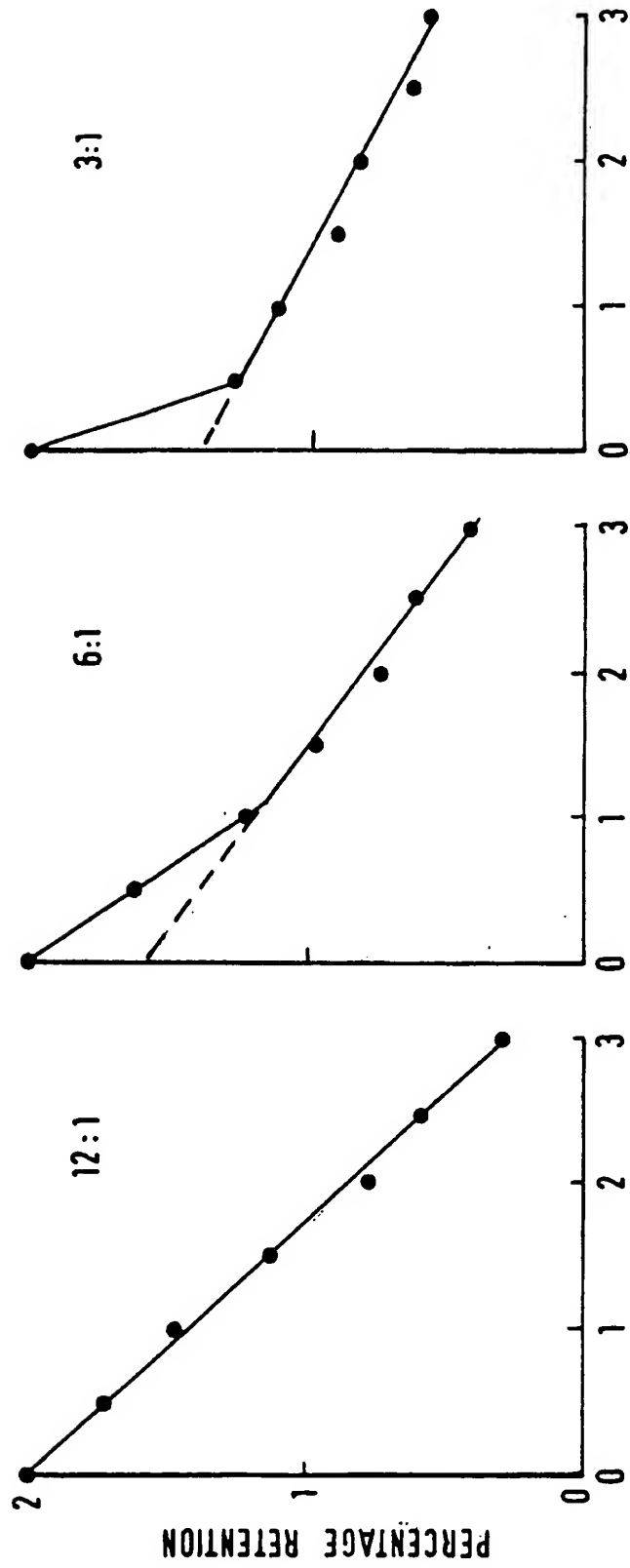


FIG.1



TIME (hours)

FIG. 2

